active materials, including proteins, for compounds which are inhibitors or activators of GLUTX activity

Compounds identified via assays such as those described herein may be useful, for example, in elaborating the biological function of GLUTX and for the treatment of disorders associated with aberrant GLUTX activity or expression. Assays for testing the effectiveness of compounds identified with the above-described techniques are discussed below.

In vitro systems may be designed to identify compounds capable of interacting with GLUTX (or a domain of GLUTX). Compounds identified may be useful, for example, in modulating the activity of wild type and/or mutant GLUTX; may be useful in elaborating the biological function GLUTX; may be utilized in screens for identifying compounds that disrupt normal GLUTX interactions; or may in themselves disrupt such interactions.

The principle of the assays used to identify compounds that bind to GLUTX involves preparing a reaction mixture of GLUTX (or a domain thereof) and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. The GLUTX species used can vary depending upon the goal of the screening assay. In some situations it is preferable to employ a peptide corresponding to a domain of GLUTX fused to a heterologous protein or polypeptide that affords advantages in the assay system (*e.g.*, labeling, isolation of the resulting complex, etc.) can be utilized.

The screening assays can be conducted in a variety of ways. For example, one method to conduct such an assay involves anchoring GLUTX protein, polypeptide, peptide or fusion protein or the test substance onto a solid phase and

detecting GLUTX/test compound complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, the GLUTX reactant may be anchored onto a solid surface, and the test compound, which is not anchored, may be labeled, either directly or indirectly.

In practice, microtiter plates may conveniently be utilized as the solid phase. The anchored component may be immobilized by non-covalent or covalent attachments. Non-covalent attachment may be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be immobilized may be used to anchor the protein to the solid surface. The surfaces may be prepared in advance and stored.

In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (*e.g.*, by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously non-immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously non-immobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; *e.g.*, using a labeled antibody specific for the previously non-immobilized component (the antibody, in turn, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted

components, and complexes detected; *e.g.*, using an immobilized antibody specific for GLUTX protein, polypeptide, peptide or fusion protein or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for the other component of the possible complex to detect anchored complexes.

Alternatively, cell-based assays can be used to identify compounds that interact with GLUTX. To this end, cell lines that express GLUTX, or cell lines that have been genetically engineered to express GLUTX can be used.

XIV. Assays for Compounds that Interfere with the Interaction Between GLUTX and a Protein Binding Partner

Proteins that interact with the GLUTX are referred to, for purposes of this discussion, as "binding partners". Such binding partners can be involved in regulating GLUTX activity. Therefore, it is desirable to identify compounds that interfere with or disrupt the interaction of such binding partners with GLUTX. Such compounds may be useful in regulating the activity of the GLUTX and treating disorders associated with aberrant GLUTX activity.

The basic principle of the assay systems used to identify compounds that interfere with the interaction between the GLUTX and binding partner or partners involves preparing a reaction mixture containing GLUTX protein, polypeptide, peptide or fusion protein and the binding partner under conditions and for a time sufficient to allow the two to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound may be initially included in the reaction mixture, or may be added at a time subsequent to the addition of the GLUTX moiety and its